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Hormonal contraceptive use, pregnancy and parity, and the risk of cervical intraepithelial neoplasia 3 among oncogenic HPV DNA-positive women with equivocal or mildly abnormal cytology

Philip E. Castle[1]*, Joan L. Walker[2], Mark Schiffman[1] and Cosette M. Wheeler[3]

[1]*Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, MD, USA*
[2]*University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA*
[3]*Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology, University of New Mexico Health Sciences Center, School of Medicine, Albuquerque, NM, USA*

Oral contraceptive (OC) use, hormonal contraceptive use and multiparity are potential risk factors for cervical precancer, cervical intraepithelial neoplasia 3 (CIN3), but a limited number of studies have adequately accounted for possible confounding effect of oncogenic human papillomavirus (HPV) infection. To examine the relationships of these factors with CIN3, we conducted an analysis of women (n = 5,060) with minimally abnormal Pap smears who were enrolled in the ASCUS and LSIL Triage Study (ALTS), a clinical trial to evaluate management strategies. Cervical specimens collected at enrollment were tested for HPV DNA using 2 methods. Multivariate logistics regression models were used to assess associations (odds ratio [OR] with 95% confidence intervals [CI]) of the potential risk factors (*e.g.*, OC use and parity) with testing oncogenic HPV positive among controls (<CIN2) (n = 4,114) and with rigorously reviewed cases of CIN3 identified throughout the study (n = 499) among women with oncogenic HPV (n = 3,126). Only former oral contraceptive use (OR = 1.3, 95% CI = 1.0–1.7) was associated marginally with having an oncogenic HPV infection among controls. Restricted to women with oncogenic HPV, current injectable hormonal contraceptive users were at an elevated risk of CIN3 (OR = 1.6, 95% CI = 1.2–2.1) compared to women who never used them. Similarly, restricted to women with HPV16 infection, current users of injectable contraceptives were at a marginally elevated risk of CIN3 (OR = 1.5, 95% CI = 1.0–2.3) compared to women who never used them. Oral contraceptive use, Norplant (implantable hormonal contraceptive) use, a history of pregnancy, age at first pregnancy, lifetime numbers of pregnancies and lifetime numbers of live births were not associated with CIN3. We conclude that only current injectable hormonal contraceptive use slightly elevated the risk (~50%) of CIN3 in this young and low parity population of women with oncogenic HPV and minimally abnormal Pap smears but further confirmation of this relationship is needed.
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Key words: cervical intraepithelial neoplasia; human papillomavirus (HPV); triage; cofactors; hormonal contraceptive use; pregnancy; parity

Cervical infections by ~15 human papillomavirus (HPV) types (oncogenic HPV) cause virtually all cases of cervical cancer,[1–3] which is the second or third most common cancer in women.[4] Globally, HPV is perhaps the most common sexually transmitted infection[5] although there can be tremendous regional variability in HPV prevalence even in adjacent countries that share similar ethnicity.[6] Most women clear HPV infections, even oncogenic HPV infections, within 2 years with or without accompanying HPV-induced cytologic or histopathologic abnormalities. Uncommonly, some oncogenic HPV infections persist, and women with persisting infection are at an elevated risk of developing cervical intraepithelial neoplasia 3 (CIN3) and cervical cancer.[7]

A number of secondary risk factors (HPV cofactors) for development of CIN3 or cancer from cancer-associated (oncogenic) HPV infection have been suggested based on epidemiologic studies, including long duration oral contraceptive (OC) use[8,9] and multiparity.[10,11] Oral contraceptives may increase the risk of CIN3 or cancer by increasing HPV viral expression *via* hormone responsive elements in the viral genome.[12] Parity could increase the risk of CIN3 or cancer by a similar hormonal-related mechanism as OC use or *via* local tissue damage that results in the release of genotoxic cellular oxidative and nitrative stresses.[13]

To examine the association of hormonal contraceptive use, including OC use and parity in the development of CIN3, cervical precancer, in young women, we undertook an analysis of oncogenic HPV DNA-positive women with minimally abnormal Pap tests recruited into the ASCUS (atypical squamous cells of unknown significance) LSIL (low-grade squamous intraepithelial lesion) Triage Study (ALTS),[14–18] a 2-year randomized prospective trial to evaluate clinical management strategies. ALTS included thorough disease and HPV assessment as the result of an intensive follow-up of patients, rigorous pathology review and dual HPV DNA testing.

Material and methods

Study design and population

ALTS was a randomized trial conducted by the National Cancer Institute (National Institutes of Health, Rockville, MD) comparing 3 triage strategies for women with ASCUS or LSIL: immediate colposcopy (IC arm), colposcopy referral for a positive test for oncogenic HPV (or the rare occurrence of repeat high-grade squamous intraepithelial lesion cytology associated with a negative test) (HPV arm), and conservative management (CM arm), the latter based on a program of repeat cytology with referral only for high-grade squamous intraepithelial lesion (HSIL). Design, meth-

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The ALTS Group is composed of the members listed above, as well as D. Solomon, M. Schiffman, P. Castle, S. Wacholder, National Cancer Institute, Bethesda, MD; E.E. Partridge, L. Kilgore, S. Hester, University of Alabama at Birmingham, AL; J.L. Walker, G.A. Johnson, A. Yadack, University of Oklahoma, Oklahoma City, OK; R.S. Guido, K. McIntyre-Seltman, R.P. Edwards, J. Gruss, Magee-Women's Hospital of the University of Pittsburgh Medical Center Health System, Pittsburgh, PA; N.B. Kiviat, L. Koutsky, C. Mao, J.M. Haug, University of Washington, Seattle, WA; Colposcopy Quality Control Group: D. Ferris, Medical College of Georgia, Augusta, GA; J.T. Cox, University of California at Santa Barbara, Santa Barbara, CA; L. Burke, Beth Israel Deaconess Medical Center Hospital, Boston, MA; HPV Quality Control Group: C.M. Wheeler, C. Peyton-Goodall, University of New Mexico Health Sciences Center, Albuquerque, NM; M.M. Manos, Kaiser Permanente, Oakland, CA; Pathology Quality Control Group: R.J. Kurman, D.L. Rosenthal, Johns Hopkins Hospital, Baltimore, MD; M.E. Sherman, The National Cancer Institute, Rockville, MD; M.H. Stoler, University of Virginia Health Science Center, Charlottesville, VA; J. Rosenthal, M. Dunn, J. Quarantillo, D. Robinson, Westat, Coordinating Unit, Rockville, MD; Quality of Life Group: D.M. Harper, Chair of ALTS Quality of Life Group, Dartmouth Medical School; A.T. Lorincz, Digene Corporation, Gaithersburg, MD; B. Kramer, Information Management Services, Inc., Silver Spring, MD.

***Correspondence to:** Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd. Room 7074, EPS MSC 7234, Bethesda, MD 20892-7234. Fax: +301-402-0916.
E-mail: castlep@mail.nih.gov

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ods, and primary results of ALTS have been published in detail elsewhere.[14–18] Briefly, women with ASCUS or LSIL cytology were recruited to participate in the study at 4 clinical centers: University of Alabama at Birmingham (Birmingham, AL), Magee-Women's Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, PA), the University of Oklahoma (Oklahoma City, OK) and the University of Washington (Seattle, WA). NCI and local institutional review boards approved the study. A total of 5,060 women who were eligible and provided informed consent were enrolled in the study from November 1996 to December 1998: 3,488 women with ASCUS cytology (mean age = 28.8 years old, median age = 26 years old, age range = 18–81 years old) and 1,572 with LSIL cytology (mean age = 24.8 years old, median age = 23 years old, age range = 18–68 years old). Routine follow-up and exit visits concluded in January 2001.

At enrollment, women in each arm received the same enrollment pelvic examination with collection of 2 cervical specimens, the first in PreservCyt for ThinPrep cytology (Cytyc, Boxborough, MA) and the second in specimen transport medium (STM; Digene, Gaithersburg, MD). Each ALTS participant was interviewed at enrollment and during follow-up to collect information on demographic, lifestyle, and medical history; there was no validation of the responses. Women exiting the study underwent a colposcopic evaluation; >80% of participants underwent an exiting exam and a colposcopic evaluation. We refer readers to other references for details on randomization, examination procedures, patient management, and laboratory and pathology methods.[14,15,18]

HPV DNA testing

Hybrid Capture 2 (Digene) using the probe set B (henceforth, referred to as HC2) is a DNA test for 13 oncogenic HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). After liquid-based, ThinPrep (Cytyc) cytology slides were prepared, 4-mL aliquots of the residual in the PreservCyt vials were used for HPV DNA testing by HC2. Signal strengths in relative light units (RLU) were compared to 1 pg/ml HPV type 16 DNA-positive controls (RLU/PC). The Food and Drug Administration-approved 1.0 RLU/PC (~1 pg/ml) was used as the threshold for a positive result.[19] Of the 5,060 women enrolled into ALTS, we had valid HC2 results on 4,819 (95.2%).

We also used L1 consensus primer PGMY09/11 PCR amplification and reverse-line blot hybridization for type-specific detection[20] on cervical specimens collected into specimen transport medium (STM; Digene) from each patient. Specimens were thawed, and a 150 uL aliquot was digested by adding 7.5 μL of digestion solution (20 mg/mL proteinase K, 10% laureth-12, 20 mM Tris, and 1 mM EDTA [pH 8.5]) and incubating at 60°C for 1 hr. DNA was precipitated by adding 1.0 mL of absolute ethanol containing 0.5 M ammonium acetate, incubating the mixture overnight at −20°C, and pelleted by centrifugation (30 min × 13,000*g*). The crude DNA pellet was dried overnight at room temperature and then suspended in 50 μL of 20 mM Tris and 1 mM EDTA (pH 8.5).

We amplified 5 μL of each crude DNA pellet by using the PGMY09/11 L1 consensus primer system and AmpliTaq gold polymerase (Perkin Elmer, Wellesley, MA). Amplifications were done in a thermal cycler (model 9600; Perkin Elmer) using the following algorithm: 9-min AmpliTaq gold activation at 95°C followed by 40 cycles of 1-min denaturation at 95°C, 1-min annealing at 55°C, and 1-min extension at 72°C, and a 5-min final extension at 72°C.

Reverse line blotting using HPV genotyping strips (Roche Molecular Systems, Alameda, CA) was used to detect 27 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–59, 66, 68, 73 [PAP238A], 82 [W13b], 83 [PAP291] and 84 [PAP155]) and a β-globin internal control. For approximately 3,000 women, we tested for 11 additional non-oncogenic genotypes (61, 62, 64, 67, 69-72, 81, 82 subtype [IS39] and 89 [CP6108]). Of the 5,060 women enrolled into ALTS, we had valid PCR tests on 4,915 (97.1%).

HPV classification

Using HC2 and PCR data, we classified HPV DNA status as positive or negative for oncogenic types[1]: oncogenic HPV positive if positive by HC2 or by PCR for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68; otherwise, negative for oncogenic HPV. Among the women negative for oncogenic HPV, we classified as non-oncogenic HPV positive those who had a positive PCR result for any HPV type other than the 13 oncogenic types listed above. We conservatively re-classified women (n = 202) as having a non-oncogenic HPV type if they were HC2 positive but PCR negative for oncogenic types and positive for either HPV6, 53, 66, 67, 70 and 81, recognizing that HC2 occasionally cross-reacts with these types especially in cervical specimens from women with cytologic abnormalities.[21] Of 5,060 women enrolled into ALTS, 5,052 (99.8%) women had at least one test result and 4,682 (92.5%) had both tests; women with only one HPV test result were classified accordingly using the results available.

Pathology

Clinical management was based on the clinical center pathologists' cytologic and histologic diagnoses. In addition, all referral smears, ThinPreps and histology slides were sent to the Pathology QC group (QC pathology) based at Johns Hopkins Hospital for review and secondary diagnoses.

Our outcome of interest was CIN3 cumulatively detected either at enrollment or during the 2-year follow-up as diagnosed by the QC pathology review. We excluded the 7 cancer cases from these analyses but their inclusion did not appreciably change estimates of risk. We used this rigorous definition of cases in recognition that CIN3 detected within 2 years of an HPV DNA-positive test is more likely to be a missed prevalent case than a true incident case, given that a single colposcopic evaluation with biopsy and histologic evaluation is not perfectly sensitive for detection of CIN3,[16] and CIN3 rarely develops from an HPV infection within 2 years. In contrast, cervical intraepithelial neoplasia 2 (CIN2) is a poorly reproducible diagnosis[22] that may represent an admixture of CIN1 and CIN3. We therefore included CIN2 into the multivariate models (described below) as an intermediate outcome, excluded from the primary case definition (CIN3) and from controls (women with oncogenic HPV and <CIN2), thereby creating a conceptual "buffer zone" between infection and CIN3. In this analysis restricted to women with oncogenic HPV (n = 3,126), 499 of 535 (93.3%) CIN3 and 361 of 396 (91.2%) CIN2 diagnosed in ALTS were included, demonstrating the extraordinarily strong relationship between oncogenic HPV detection and diagnoses of ≥CIN2 (*i.e.*, only 7.7% of ≥CIN2 detected within 2 years of recruitment were HPV DNA negative at enrollment).

Analysis

Standard contingency table methods, with Pearson χ^2 tests or, when appropriate, the Mantel extension test for trend, were used to assess possible univariate associations of categorical variables with being oncogenic HPV DNA-positive in controls (*i.e.*, <CIN2) (n = 3710) and with CIN2 (n = 361) or CIN3 (n = 499) (*vs.* <CIN2) among oncogenic HPV DNA-positive women (n = 3,126). Stepwise logistic regression modeling was used to calculate odds ratios (OR) and 95% confidence intervals (CI) as measures of association of pregnancy, parity and hormonal contraceptive use with detection of oncogenic HPV DNA in controls. Stepwise multinomial logistic regression modeling was used to calculate OR and 95% CI as measures of association of pregnancy, parity and hormonal contraceptive use with diagnoses of CIN3 or CIN2 compared to controls.

Oral contraceptive, injectable contraceptive and implantable hormonal (Levonorgestrel) contraceptive (Norplant; Wyeth-Ayerst Laboratories, Philadelphia, PA) use were defined never,

TABLE I – ASSOCIATIONS OF HORMONAL CONTRACEPTIVES AND PREGNANCY WITH DETECTION OF ONCOGENIC HPV DNA AMONG WOMEN WHO DID NOT HAVE CIN2 OR WORSE DIAGNOSIS DURING THE 2-YEAR STUDY PERIOD[1]

Exposure	N (%)	N− (%)	N+ (%)	OR[3] (95% CI)
Oral contraceptives				
Never	1,711 (42)	697 (52)	814 (36)	1.0 (ref.)
Former	658 (16)	137 (10)	425 (19)	1.3 (1.0–1.7)[2]
Current	1,729 (42)	502 (38)	1,015 (45)	0.97 (0.81–1.1)
Injectable contraceptives				
Never	3,210 (78)	1,135 (85)	1,668 (74)	1.0 (ref.)
Former	366 (9)	75 (6)	256 (11)	1.2 (0.86–1.5)
Current	532 (13)	129 (10)	337 (15)	1.0 (0.81–1.3)
Norplant				
Never	4,007 (97)	1,318 (98)	2,198 (97)	1.0 (ref.)
Former	67 (2)	16 (1)	41 (2)	0.91 (0.49–1.7)
Current	42 (1)	9 (1)	24 (1)	0.79 (0.34–1.9)
Pregnancy				
Never	1,180 (29)	285 (21)	720 (32)	1.0 (ref.)
Ever	2,938 (71)	1,058 (79)	1,544 (68)	0.88 (0.73–1.1)

[1]Odds ratio with 95% CI from a multivariate logistic regression model comparing oncogenic HPV positive *vs*. HPV negative women. N, total number of women exposed; N−, number of HPV-negative women exposed; N+, number of oncogenic HPV-positive women exposed. Women with non-oncogenic HPV contributed to N but were excluded from the models.–[2]Lower or upper confidence bound does not include 1.00.–[3]Adjusted for age, study center and recent/lifetime numbers of sexual partners (0 and 0–2, 0 and ≥3, 1 and 0–2, 1 and ≥3, ≥2 and 0–2, ≥2 and ≥3).

former (no use within the past month), and current. Information on duration of use and details regarding the type of injectable or oral contraceptive were not collected. Because we considered all cases of CIN3 to be prevalent, including those detected during follow-up that were missed at baseline due to the insensitivity of cytology (in the CM arm) and colposcopy (in the IC arm and in women who tested positive by cytology and HPV testing in the CM and HPV arms, respectively), we did not consider data related to pregnancy, parity and hormonal contraceptive use collected during follow-up in these analyses. Pregnancy and parity variables included ever pregnant, age at first pregnancy (never pregnant, 12–17 years, 18–20 years, and ≥21 years), number of pregnancies (never pregnant, 1 pregnancy, 2–3 pregnancies, ≥4 pregnancies), and number of live births (never pregnant, pregnancy but no live births, 1–2 live births, ≥3 live births). Final models to examine the associations of these factors with being oncogenic HPV DNA-positive among women with <CIN2 included adjusted for age (18–19, 20–24, 25–29, 30–34 and ≥35 years), recent (within the last year) and lifetime numbers of sexual partners (0 recent/0–2 lifetime, 0 recent/≥3 lifetime, 1 recent/0–2 lifetime, 1 recent/≥3 lifetime, ≥2 recent/0–2 lifetime and ≥2 recent/≥3 lifetime), and study center; other covariates did not appreciably alter the associations of these exposures and HPV DNA detection. Final models to examine associations of these factors with CIN3 and with CIN2 included adjusted for education (<high school diploma, high-school diploma and post high-school education <college degree, and a college degree or more education), whether a woman had an HPV16 infection, smoking (never, former, current), and age. Inclusion of other variables, *e.g*., age at sexual debut and number of HPV types detected by PCR, did not appreciably alter our estimates of associations of hormonal contraception, pregnancy and parity with CIN2 or CIN3.

Results

Oral contraceptive use

At enrollment, 42.9% and 16.5%, of women in ALTS were current and former users of OC, respectively; among oncogenic HPV DNA-positive women, 45.4% and 18.6% of women were current and former users of oral contraceptives, respectively. Former, but not current, OC use was weakly associated with being oncogenic HPV DNA-positive (OR = 1.3, 95% CI = 1.0–1.7) compared to never users in a multivariate model (Table I).

Among oncogenic HPV DNA-positive women, current or former OC use was not associated with CIN2 or with CIN3 compared to never users (Table II). Similarly, current or former OC use was not associated with CIN2 or with CIN3 compared to never users in HPV16 DNA-positive women (Table III).

Injectable contraceptive use

At enrollment, 14.5% and 9.3%, of women in ALTS were current and former users of injectable contraceptives, respectively; among oncogenic HPV DNA-positive women, 16.5% and 11.3% of women were current and former users of injectable contraceptives, respectively. Current and former injectable contraceptive use were not associated being HPV DNA-positive compared to never users in a multivariate model (Table I).

Among oncogenic HPV DNA-positive women, current injectable contraceptive use was associated with CIN2 (OR = 1.4, 95% CI = 1.1–1.9) and with CIN3 (OR = 1.6, 95% CI = 1.2–2.1) compared to never users (Table II). In HPV16 DNA-positive women, current injectable contraceptive use was marginally associated with CIN3 (OR = 1.5, 95% CI = 1.0–2.3), but not with CIN2, compared to never users (Table III).

Norplant use

At enrollment, 1.1% and 1.9%, of women in ALTS were current and former users of Norplant, respectively; among oncogenic HPV DNA-positive women, 1.1% and 2.2% of women were current and former users of Norplant, respectively. Current and former Norplant use was not associated being oncogenic HPV DNA-positive compared to never users in a multivariate model (Table I).

Among oncogenic HPV DNA-positive women, former Norplant use was associated with CIN2 (OR = 2.0, 95% CI = 1.0–3.8), but not with CIN3, compared to never users (Table II). In HPV16 DNA-positive women, former Norplant use was associated with CIN2 (OR = 4.7, 95% CI = 1.1–20), but not with CIN3 compared to never users (Table III).

Pregnancy and parity

In ALTS, 71.2% women and 68.9% oncogenic HPV DNA-positive women had ever been pregnant at the time of enrollment. The median and mean ages of first pregnancy for all women were 18.0 years and 19.2 years and for women with oncogenic HPV infection were 18.0 years and 18.6 years. The median and mean numbers of pregnancies for all women who were ever pregnant were 2.0 and 2.4 and for women with oncogenic HPV infection were 2.0 and 2.2. The median and mean numbers of live births for all women who were ever pregnant were 1.0 and 1.6 and for women

TABLE II – ASSOCIATION OF HORMONAL CONTRACEPTIVES AND PREGNANCY WITH CIN2 AND CIN3 *VS.* WOMEN WITH <CIN2 AMONG ONCOGENIC HPV DNA POSITIVE WOMEN[1]

	All		<CIN2		CIN2[2]			CIN3[2]		
	N	%	N	%	N	%	OR[3] (95% CI)	N	%	OR[3] (95% CI)
Oral contraceptive use										
Never	1,123	36	814	36	124	26	1.0 (ref.)	181	36	1.0 (ref.)
Former	579	19	425	19	70	21	1.1 (0.76–1.4)	84	17	0.86 (0.63–1.2)
Current	1,415	45	1,015	45	164	53	1.1 (0.84–1.4)	233	47	1.0 (0.79–1.3)
Injectable contraceptive use										
Never	2,256	72	1,668	74	246	69	1.0 (ref.)	337	68	1.0 (ref.)
Former	353	11	256	11	41	11	1.1 (0.74–1.5)	55	11	1.1 (0.77–1.5)
Current	515	16	337	15	72	20	1.4 (1.1–1.9)[4]	105	21	1.6 (1.2–2.1)[4]
Norplant use										
Never	3,026	97	2,198	97	343	95	1.0 (ref.)	479	96	1.0 (ref.)
Former	69	2	41	2	13	4	2.0 (1.0–3.8)[4]	14	3	1.2 (0.61–2.5)
Current	34	1	24	1	5	1	1.2 (0.47–3.3)	5	1	0.72 (0.25–2.0)
Ever pregnant										
Never	973	31	720	32	108	30	1.0 (ref.)	144	29	1.0 (ref.)
Ever	2,158	69	1,544	68	253	70	1.1 (0.85–1.4)	355	71	1.1 (0.83–1.4)
Age at 1st pregnancy (years old)										
Never pregnant	973	31	720	32	108	30	1.0 (ref.)	144	29	1.0 (ref.)
< 18	863	28	589	26	111	31	1.2 (0.89–1.7)	161	32	1.2 (0.90–1.6)
18–20	834	27	614	27	94	26	1.1 (0.77–1.4)	123	25	0.92 (0.69–1.2)
≥ 21	461	15	341	15	48	13	1.0 (0.68–1.5)	71	14	1.1 (0.74–1.5)
Number of pregnancies										
Never pregnant	973	31	720	32	108	30	1.0 (ref.)	144	29	1.0 (ref.)
1 pregnancy	859	27	622	28	96	27	1.0 (0.76–1.4)	139	28	1.0 (0.76–1.3)
2–3 pregnancies	959	31	672	30	116	32	1.2 (0.89–1.7)	168	34	1.2 (0.90–1.6)
≥ 4 pregnancies	339	11	249	11	41	11	1.2 (0.75–1.8)	48	10	0.87 (0.57–1.3)
Number of live births										
Never pregnant	973	31	720	32	108	30	1.0 (ref.)	144	29	1.0 (ref.)
No live births	444	14	332	15	47	13	0.93 (0.64–1.4)	63	13	0.82 (0.58–1.2)
1–2 births	1,368	44	963	43	161	45	1.2 (0.88–1.6)	241	48	1.2 (0.92–1.6)
≥ 3 births	346	11	249	11	45	13	1.3 (0.85–2.1)	51	10	1.0 (0.65–1.5)

[1]The number (N) and percentage (%) of oncogenic HPV positive women are included for all women, control women (<CIN2), women diagnosed with CIN2, and women diagnosed with CIN3. Odds ratios (ORs) with 95% CI from multinomial logistic regression models comparing women with a CIN2 or CIN3 diagnosis to women with a <CIN2 diagnosis.–[2]Includes all cases diagnosed at enrollment, during the 2-year follow-up and at the exit colposcopy.–[3]Adjusted for detection of HPV 16 DNA, education, age, and smoking status.–[4]Indicates OR for which the lower or upper confidence bound does not include 1.00.

with oncogenic HPV infection were 1.0 and 1.4. Pregnancy and parity variables were not associated with being oncogenic HPV DNA-positive in a multivariate model (Table I).

No measures of pregnancy or parity were associated with CIN2 or with CIN3 in women with oncogenic HPV (Table II) or with HPV16 (Table III). Pregnancy during the follow-up phase of ALTS was not associated with CIN3 (data not shown).

Discussion

Oral contraceptive use

Interestingly, we did not observe any link between OC use and CIN3, which conflicts with earlier, prominent reports linking OC use and cervical cancer.[8,9] There were several differences and limitations in our study compared to other studies that may help to explain this discrepancy.

CIN3 was our primary outcome whereas other studies have used cancer as the outcome. Current opinion[23] suggests that OC use, acting *via* steroid hormone response elements in the HPV genome, increases viral expression of E6 and E7. It is therefore conceivable that OC use could promote the transition of CIN3 to invasive cancer perhaps by increasing expression of these oncoproteins. Anecdotally, 3 of 7 women diagnosed with cancer were current OC users and all 3 were under the age of 28 years, which is an unusually young age for cervical cancer diagnoses, whereas the other 4 women with cancer were never users and over the age of 35 years. We did not collect information on the duration of OC use, and only long-duration OC use, not ever use, has been linked with risk of cancer.[8,9] Therefore, we may not have identified the OC users at the greater risk of CIN3. Finally, our study population was young (median age of 23 years, 25–75% interquartile range of 20–28 years and 95% under the age of 40 years) compared to these other populations in which OC use elevated the risk of cancer. It is conceivable but untested that effects of OC use on HPV-induced cancer manifest in older women; however, we did not find any elevated risk in women 35 years and older.

The studies linking OC use and risk of cervical cancer have derived mainly from populations with less intensive screening and treatment of cancer precursors than the U.S. populations in ALTS. The risk attributable OC use might be attentuated by aggressive screening, as we postulated after the similarly null results from a U.S.-based cohort study.[24] OC use may increase viral load and therefore increase the likelihood of cytologic manifestations of infection such as ASCUS and LSIL, which could lead to censoring of those about to develop CIN3. There is some evidence for this differential censoring based on OC use.[24] However, women were referred into this study for those same cytologic interpretations, making this explanation unlikely. Another possibility is that by evaluating the association of OC with CIN3 in a population with mild or equivocal cytologic abnormalities, typically caused by a productive viral infection, we may have inadvertently matched on the causal effect of OC use *i.e.*, OC increase viral expression *via* hormone responsive elements.

A final possibility is that the doses of contraceptive hormones women were exposed to in this study differs from the doses experienced in these other populations. Compositions of oral contraceptives have decreased hormone doses over time as it became apparent that these smaller doses were sufficient for the desired contraceptive effect. Thus, exposures to the hormones may differ

TABLE III – ASSOCIATION OF HORMONAL CONTRACEPTIVES, PARITY AND PREGNANCY WITH CIN2 AND CIN3 *VS.* WOMEN WITH <CIN2 AMONG HPV 16 DNA-POSITIVE WOMEN[1]

	All		<CIN2		CIN2[2]			CIN3[2]		
	N	%	N	%	N	%	OR[3] (95% CI)	N	%	OR[3] (95% CI)
Oral contraceptive use										
Never	275	34	151	35	124	35	1.0 (ref.)	93	33	1.0 (ref.)
Former	143	17	70	16	70	20	1.3 (0.70–2.5)	51	18	1.2 (0.74–1.9)
Current	404	49	205	48	164	46	1.2 (0.73–2.1)	140	49	1.2 (0.83–1.7)
Injectable contraceptive use										
Never	597	72	323	76	79	73	1.0 (ref.)	191	68	1.0 (ref.)
Former	94	11	44	10	14	13	1.2 (0.62–2.4)	35	13	1.4 (0.87–2.4)
Current	134	16	61	14	15	14	0.92 (0.49–1.7)	57	20	1.5 (1.0–2.3)[4]
Norplant use										
Never	802	97	419	98	103	95	1.0 (ref.)	275	96	1.0 (ref.)
Former	16	2	4	1	4	4	4.7 (1.1–20)[4]	7	2	1.6 (0.44–5.9)
Current	10	1	5	1	2	2	1.5 (0.28–8.0)	3	1	0.72 (0.16–3.1)
Ever pregnant										
Never	277	34	148	35	43	39	1.0 (ref.)	85	30	1.0 (ref.)
Ever	551	67	280	65	66	61	0.91 (0.57–1.5)	200	70	1.0 (0.71–1.4)
Age at 1st pregnancy (years old)										
Never pregnant	277	34	148	35	43	39	1.0 (ref.)	85	30	1.0 (ref.)
< 18	229	28	117	27	26	24	0.78 (0.44–1.5)	84	29	0.93 (0.61–1.4)
18–20	207	25	100	23	29	27	1.1 (0.61–2.0)	75	26	1.1 (0.69–1.6)
≥ 21	115	14	63	15	11	10	0.90 (0.40–2.0)	41	14	1.1 (0.66–1.8)
Number of pregnancies										
Never pregnant	277	34	148	35	43	39	1.0 (ref.)	85	30	1.0 (ref.)
1 pregnancy	250	30	133	31	33	30	0.88 (0.52–1.5)	83	29	0.96 (0.64–1.4)
2–3 pregnancies	225	27	103	24	26	24	1.0 (0.56–1.9)	93	33	1.2 (0.78–1.9)
≥ 4 pregnancies	75	9	43	10	7	6	0.77 (0.29–2.0)	24	8	0.68 (0.36–1.3)
Number of live births										
Never pregnant	277	34	148	35	43	39	1.0 (ref.)	86	30	1.0 (ref.)
No live births	134	16	76	18	16	15	0.74 (0.39–1.4)	41	14	0.83 (0.51–1.3)
1–2 births	339	41	157	37	42	39	1.0 (0.61–1.7)	137	48	1.2 (0.81–1.7)
≥ 3 births	78	9	47	11	8	7	0.89 (0.35–2.2)	22	8	0.60 (0.31–1.2)

[1]The number (N) and percentage (%) of HPV16 positive women are included for all women, control women (<CIN2), women diagnosed with CIN2, and women diagnosed with CIN3. Odds ratio with 95% CI from multinomial logistic regression models comparing women with a CIN2 or CIN3 diagnosis to women with a <CIN2 diagnosis.–[2]Includes all cases diagnosed at enrollment, during the 2-year follow-up and at the exit colposcopy.–[3]Adjusted for education, age, and smoking status.–[4]Indicates OR for which the lower or upper confidence bound does not include 1.00.

substantially between studies, and the duration of use effect observed in these other populations may also be a surrogate for these higher dose oral contraceptives.

Injectable contraceptive use

We found that oncogenic HPV infected women with minimally abnormal Pap smears who were using injectable contraception at enrollment were at a ~50% greater risk of having CIN3 within 2 years compared to woman who did not use it. This association was also observed in HPV16 infected women. We did not find injectable contraception use associated with having an oncogenic HPV infection, abrogating concerns that its use was a surrogate for HPV acquisition, which could have explained the elevated risk. Other studies,[25,26] however, have not found an association of injectable contraceptive use and cancer. Therefore, our observation is unsubstantiated and could be a false positive finding due to multiple comparisons. Confirmatory evidence is needed before any meaning conclusions can be made about injectable contraception and the risk of CIN3.

Norplant use

Norplant was not associated with CIN3 but former use was associated with CIN2; however this finding was based on small numbers and CIN2 is a poorly reproducible diagnosis.[22] Given the lack of association with CIN3, we argue that the association of Norplant and CIN2 maybe a chance finding and not relevant to the risk of developing a true cervical precancerous lesion (CIN3).

Pregnancy and parity

We also did not observe any relationship of pregnancy or parity with the risk of CIN2 or CIN3, contrary to a multi-centric, international study,[10] which found an elevated risk of cervical cancer among multi-parous women, with a 4-fold increase in risk in women with 7 or more births. In ALTS, only 11 women reported having 7 or more births. This discordance may be the result of our study being underpowered, the effects of different outcomes (CIN3 *vs.* cancer), or the result of (greater) differential censoring of HPV-infected pregnant women in our study compared to the multi-centric study, perhaps attributable to increased pre-enrollment surveillance in women enrolled in ALTS compared to women in the other study.

Conclusions

We conclude that hormonal contraceptives, pregnancy, and parity had little or no impact on having an oncogenic HPV infection or on the development of CIN3 in this young, low parity, oncogenic HPV infected women with minimally abnormal Pap smears. Although not a representative population, cytologic changes among women with HPV infections are common and restricting analysis to those who have oncogenic HPV controls for possible confounding due to the primary risk factor. We attempted to minimize possible misclassifications of both HPV status and histopathologic outcomes by utilizing dual HPV testing and rigorous pathology review, respectively, to accurately assess associations with CIN3. We assumed that cases of CIN3 diagnosed during the 2-year follow-up were missed prevalent disease but there was likely some small percentage of incident cases. Many women changed their contraceptive choices during ALTS (data not shown) and thus we may have biased our estimates of the risk of using hormonal contraceptives toward the null by this rigorous definition of prevalent disease. Use of injectable contraceptives was found to slightly increase the risk of CIN3 but use of other

hormonal contraceptives was not, casting doubt regarding the veracity of this association. Differences either in the composition, peak exposure, or overall exposure to contraceptive hormones between these contraceptive choices could theoretically explain these findings. We cannot, therefore, rule out that the use of injectable contraceptives may contribute to risk of CIN3 in oncogenic HPV-infected women.

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